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Identification, classification, and discrimination of agave syrups from natural sweeteners by infrared spectroscopy and HPAEC-PAD

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ABSTRACT

Agave syrups are gaining popularity as new natural sweeteners. Identification, classification and discrimination by infrared spectroscopy coupled to chemometrics (NIR-MIR-SIMCA-PCA) and HPAEC-PAD of agave syrups from natural sweeteners were achieved. MIR-SIMCA-PCA allowed us to classify the natural sweeteners according to their natural source. Natural syrups exhibited differences in the MIR spectra region 1500–900 cm^{-1}. The agave syrups displayed strong absorption in the MIR spectra region 1061–1063 cm^{-1}, in agreement with their high fructose content. Additionally, MIR-SIMCA-PCA allowed us to differentiate among syrups from different *Agave* species (*Agave tequilana* and *Agave salmiana*). Thin-layer chromatography and HPAEC-PAD revealed glucose, fructose, and sucrose as the principal carbohydrates in all of the syrups. Oligosaccharide profiles showed that *A. tequilana* syrups are mainly composed of fructose (>60%) and fructooligosaccharides, while *A. salmiana* syrups contain more sucrose (28–32%). We conclude that MIR-SIMCA-PCA and HPAEC-PAD can be used to unequivocally identify and classified agave syrups.

1. Introduction

Agave syrup is the naturally sweet substance produced when agave pines are cooked. The use of food additives for adulteration or mixing agave syrup with starch, molasses, glucose, dextrin, fructose, or other sugars from sources other than agave plants are not permitted in commercial agave syrup production (Ramos, 2009). Agave syrups are in great demand as sugar substitutes because of their low glycaemic index (Foster-Powell, Holt, & Brand-Miller, 2002), antioxidant capacity (Phillips, Carlsen, & Blomhoff, 2009), and antibacterial properties (Davidson & Ortiz de Montellano, 1983). The popularity of agave syrups has led to the development of new strategies seeking to optimise agave syrup production by elaborating the syrups *via* the enzymatic hydrolysis of agave fructans instead of the traditional methods based on thermal or acid hydrolysis (García-Aguirre et al., 2009).

Vibrational spectroscopic methods [near-infrared (NIR) and mid-infrared (MIR)] in combination with chemometrics (multivariate data analysis) present a nondestructive, rapid, simple, and low-cost approach for screening samples of any type. Infrared (IR) spectroscopy has been applied to determine the presence and quantity of sugars in aqueous mixtures (Kemsley, Zhuo, Hammouri, & Wilson, 1992; Wang, Kliks, Jun, Jackson, & Li, 2010) and to authenticate the botanical and geographical origin of honey samples (Ruoff, Luginbûhl, Bogdanov, et al., 2006; Ruoff, Luginbûhl, Kûnzli, et al., 2006), allowing Irish artisanal honey to be discriminated from such honey adulterated with various sugar syrups (Kelly, Petisco, & Downey, 2006). In addition, IR spectroscopy has the potential to discriminate among and classify adulterants in maple syrups (Paradkar, Sivakesava, & Irudayaraj, 2003).

Principal components analysis (PCA) is a statistical technique that explores unsupervised pattern recognition, enabling the graphical representation of objects or variables in clusters or groups based on similarities (Cheajesadagul, Arnaudguilhem, Shiowatana, Siripinyanond, & Szpunar, 2013; Kelly et al., 2006). The aim of PCA is to express the main information contained within a larger group of variables using a smaller group of variables, defined as principal components (PCs), which describe the main sources of variation in the data. PCs are orthogonal (uncorrelated with each other), hierarchical (the first PC retains the main information of the data, the second PC retains the main information that is not included in the first PC, and so on), and calculated sequentially (Beebe, Pell, & Seasholiz, 1998; Bro & Andersson, 1998).

High-performance anion exchange chromatography with a pulsed amperometric detector (HPAEC-PAD) is recommended for carbohydrate analyses of honey samples because of its low detection limits. Carbohydrate profiles analyses are a valuable tool for characterising and classifying honeys from different botanical

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origins and geographical sources (Nozal, Bernal, Toribio, Alamo, & Diego, 2005). Indeed, HPAEC-PAD can also be used to establish carbohydrate profiles for detecting the adulteration of honey with corn sugar (Megherbi, Herbrereau, Faure, & Salvador, 2009; Morales, Corzo, & Sanz, 2008). Carbohydrate profiles combined with fructose/glucose (F/G) or maltose/isomaltose ratios have been employed to evaluate the adulteration of honey with glucose, sugar cane, or high-fructose syrups (Guler, Bakan, Nisbet, & Yavuz, 2007; Ischayek & Kern, 2006; Nozal et al., 2005).

The aim of this study was to apply NIR and MIR spectroscopic techniques in combination with chemometrics (PCA) to identify, classify, and discriminate agave syrups from other natural sweeteners. We also aimed to simultaneously assess the potential of HPAEC-PAD to establish differences in the oligosaccharide contents and profiles and the monosaccharide ratios of agave syrups and several other natural sweeteners.

2. Materials and methods

2.1. Standards

Glucose, fructose, and sucrose were acquired from Sigma–Aldrich (St. Louis, MO), maltooligosaccharides (MOS: G2–G7) from Supelco (Bellefonte, PA), and fructooligosaccharides (FOS: 1-kestose, 1-nystose and 1-kestopentaose) were obtained from Wako Pure Chemical Industries (Tokyo, Japan).

2.2. Natural syrups

A total of 43 natural sweeteners from different sources were obtained from supermarkets and convenient stores: 25 samples of agave syrup (AS1–AS25), 2 samples of corn syrup (CS1–CS2), 13 samples of honey (HB1–HB13), and 3 samples of sugar cane syrup (SCS1–SCS3). The agave syrup samples came from either *Agave tequilana* Weber blue variety (AS1–AS21) the raw material used for tequila production and hence the most economically important *Agave* species in Mexico (Aguilar-Romo, 2006) or *Agave salmiana* (AS22–AS25), which is distributed in central Mexico and used mainly for aguamiel, pulque, and mezcal production (Gentry, 1982; Martínez-Aguilar & Peña-Álvarez, 2009).

All the samples were stored at 4 °C until analysis. Immediately prior to analysis, the samples were incubated at 50 °C for 1 h, manually stirred, sonicated for 30 min to ensure homogeneity, and then maintained at room temperature (25 °C).

2.3. Physicochemical properties

The physicochemical properties of all the syrups were determined according to the specifications and test methods described in the Mexican Regulations for agave syrup, NMX-FF-110-SCFI-2008 (Ramos, 2009).

2.3.1. Total soluble solids and moisture content

The total soluble solids (°Brix) and moisture content (%*M*) were measured in an Abbe refractometer 736008 from Carl Zeiss (Jena, Germany). Two hundred microlitres of syrup were placed on the refractometer prism, and readings were taken of the °Brix scale and the refractive index. The %*M* was calculated according to the table of equivalences for refractive indexes and humidity (USDA, 1985). Before the measurements were taken, the accuracy of the refractometer was standardised with distilled water at 20 °C.

2.3.2. pH

The pH of the samples was measured on a pH meter Jenway 3510 potentiometer (Bibby Scientific Ltd., Stone, UK). Briefly, 10 g of syrup were dissolved in 75 mL of distilled water free of carbon dioxide. Two millilitres of the solution were then taken, and the pH was determined. Before the analysis, the equipment was calibrated with buffer solutions of pH 4.00 and pH 7.00 (Ramos, 2009).

2.3.3. Colour (DO 560 nm)

The colour designation of the natural syrups was determined according to the United States Standards for Grades of Extracted Honey Approved Colour Standards (USDA, 1985). In short, the light absorbance was measured at 560 nm using a glycerol solution as reference. The syrup colours were classified using the seven categories developed by USDA: water white, extra white, white, extra light amber, light amber, amber, and dark amber.

2.3.4. Statistical analysis

All analyses were carried out in triplicate and the data were expressed as means and standard deviations (SD). ANOVA analyses were performed using the Statgraphics Plus software version 5.1 (2001; StatPoint, Inc., Herndon, VA).

2.4. Infrared spectroscopy

2.4.1. Near-infrared spectroscopy

The samples were liquefied in a water bath at 50 °C for 1 h and then allowed to cool to room temperature before analysis. A 100 mg mL^{-1} solution was prepared for each sample. One millilitre of the prepared solutions was applied to the sampling plate and left to thermally equilibrate for 1 min. NIR spectra were recorded using a Paragon IdentiCheck FT-NIR spectrometer (Perkin Elmer, Beaconsfield, UK). Thirty-two scans with a resolution of 4 cm^{-1} were recorded in transmittance (%*T*) mode for each spectrum in the wavenumber range between 10,000 and 4000 cm^{-1}. Three replicate measures of each sample were taken. Spectral data collections were performed with Spectrum IdentiCheck software (Perkin Elmer).

To exclude measurement noise in the chemometric analysis, NIR spectra models were created for the spectral regions from 8000 to 4000 cm^{-1} and from 5200 to 4200 cm^{-1}, which are the dominant composition wavelength ranges of the relevant sugars (Hollung et al., 2005; Ruoff, Luginbûhl, Bogdanov, et al., 2006). The NIR analysis began with the transformation of all spectra to the absorbance mode with nine-point segment smoothing.

2.4.2. Mid-infrared spectroscopy

Fourier-transformed MIR spectra were recorded using a Perkin Elmer 1600 FT-IR Spectrometer (Perkin Elmer) equipped with a compartment horizontal attenuated total reflectance (HATR) trough top plate by use of a 45° zinc selenide (ZnSe) crystal with an 11 internal-reflections accessory (Perkin Elmer, Beaconsfield, UK). The samples were liquefied in a water bath at 50 °C for 1 h and then allowed to cool to room temperature before analysis. A 100 mg mL^{-1} solution was prepared for each sample. One millilitre was taken from each solution, applied to the flat sampling plate, and left to thermally equilibrate for 1 min. Thirty-two scans were recorded in the range between 4000 and 650 cm^{-1} at a nominal resolution of 4 cm^{-1} in transmittance mode (%*T*). Single-beam spectra of the samples were collected against an air background. Three replicate measures of each sample were taken. Spectral data collections were performed with Spectrum software (Perkin Elmer).

For the chemometric analysis, MIR spectra models were developed for carbohydrates (1185–950 cm^{-1}), proteins (1720–1480 cm^{-1}), and fatty acids (3000–2840 cm^{-1}) (Adt, Toubas, Pinon, Manfait, & Sockalingum, 2006; Kelly et al., 2006; Kemsley et al., 1992; Ruoff, Luginbûhl, Kûnzli, et al., 2006; Tewari &

Irudayaraj, 2004). All spectra were transformed to absorbance mode with nine-point segment smoothing.

2.4.3. Principal component analysis

PCA was carried out using Spectrum Quant software (Perkin Elmer, Beaconsfield, UK) and validated with the spectra of randomly selected samples that were not included among those used to build the model.

2.5. Carbohydrate profiles and contents

2.5.1. Thin layer chromatography

An aliquot of 1 μL of syrup solution (100 mg mL^{-1}) was applied to a silica gel TLC plate with aluminium support. The TLC plate was developed in a solvent system of butanol/propanol/water (Kanaya, Chiba, & Shimomura, 1978) and sprayed with aniline/diphenylamine/phosphoric acid reagent in acetone for carbohydrate visualisation (Anderson, Li, & Li, 2000).

2.5.2. High performance anion exchange chromatography with pulsed amperometric detection

The types and amounts of carbohydrates in the natural sweeteners were analysed and quantified by HPAEC-PAD according to the method established by Mellado-Mojica and López (2012, 2013) in a Dionex ICS-3000 ion chromatograph (Dionex, Sunnyvale, CA) with a CarboPac PA-100 guard column (4 mm × 50 mm) and a CarboPac-PA100 analytical column (4 mm × 250 mm). The syrups were diluted to a concentration of 0.2 mg mL^{-1} with deionised water (resistivity of 17 MΩ) and then filtered through a nylon membrane with 0.45-μm pores before injection. Twenty-five microlitres of each diluted sample was injected into the HPAEC. Carbohydrates were separated using a gradient of sodium acetate in 0.15 M NaOH at a flow of 0.8 mL min^{-1} and a column temperature of 25 °C. The potentials applied for detection by the amperometric pulse were E1 (400 ms), E2 (20 ms), E3 (20 ms), and E4 (60 ms) of +0.1, −2.0, +0.6, and −0.1 V, respectively.

3. Results and discussion

3.1. Physicochemical properties of the natural syrups

We determined the physicochemical parameters of the agave syrups and the other syrups (corn, sugar cane, and honey bee) to establish differences among the sweeteners based on their natural origins.

The total soluble solids, moisture, pH, and colour of the natural sweeteners were very similar regardless of the natural origin of the sweeteners (Fig. 1). The total soluble solids (°Brix) values were very similar among all the natural syrups (Fig. 1A). The agave syrups ranged from 65 to 79.5 °Brix, the corn syrups ranged from 76.5 to 77.4 °Brix, and the sugar cane syrups ranged from 69.8 to 80.1 °Brix; the honey was somewhat higher, with maximum values ranging from 80 to 84 °Brix. The moisture content (%*M*) was similar among all the sweeteners as well, ranging from 14.4% to 33% (Fig. 1B). The honey had the lowest moisture level, ranging from 14.4% to 18.2%, while the agave and sugar cane syrups had very similar moisture levels. All the natural syrups were slightly acidic, with pH values between 3.36 and 5.26 (Fig. 1C). The pH of the agave syrups ranged from pH 3.66 to pH 5.23.

The natural syrups exhibited a wide range of colours regardless of their origin. The agave syrups showed a wide range of light absorbance (0.017–3.956) and exhibited a variety of colour categories from water white to dark amber (Fig. 1D). The corn syrups exhibited only the water white colour (absorbance < 0.0945). The honey exhibited colours ranging from water white to light amber (absorbance from 0.034 to 0.652). The sugar cane syrups displayed the darkest colours, ranging from amber to dark amber (absorbance from 1.471 to 3.956).

The physicochemical properties of the agave syrups from *A. salmiana* (AS22–AS25) contrasted with those of the other agave syrups; the former had the lowest total soluble solids content, the highest moisture content, and the darkest colour among the agave samples.

Overall, the physicochemical properties of the natural sweeteners were very similar, highlighting the importance of finding useful tools to differentiate among sweeteners from different sources.

3.2. Classification of the agave syrups by infrared spectroscopic techniques

IR spectroscopic techniques combined with multivariate data analysis have been used as a nondestructive way to quantify and characterise biological samples. These techniques can rapidly provide a considerable amount of information about a sample and have been successfully used to determine components such as carbohydrates, fats, vitamins, amino acids, proteins, and moisture in

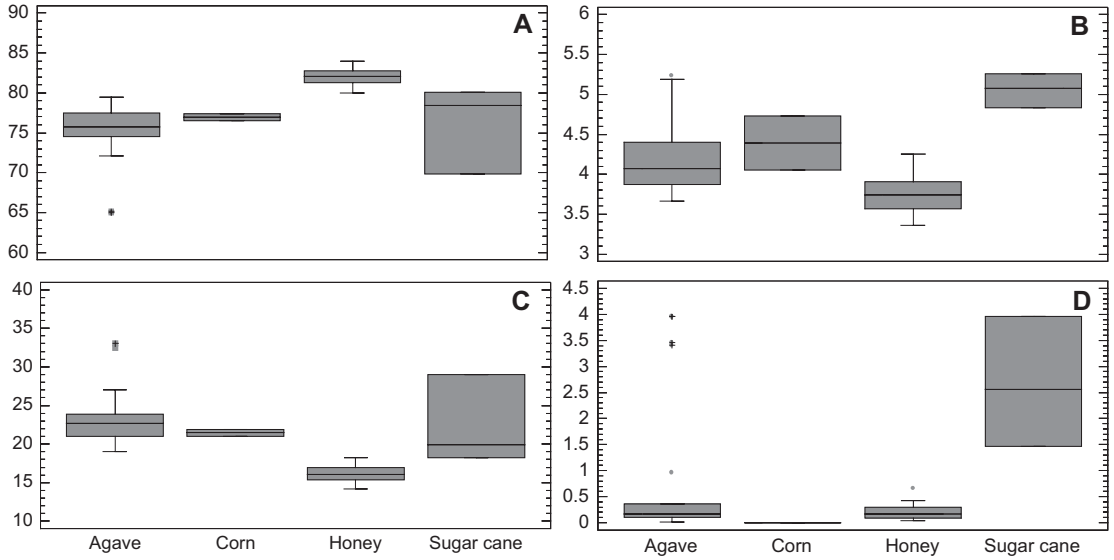

Fig. 1. Physicochemical properties of the natural sweeteners; (A) total soluble solids (°Brix); (B) pH; (C) moisture content (%*M*); (D) colour (*DO* 560 nm).

foods and agricultural products in a single analysis (Sivakesava & Irudayaraj, 2000; Tewari & Irudayaraj, 2004; Wang et al., 2010; Yang & Irudararayj, 2002).

3.2.1. Near-infrared spectroscopy

There were no significant differences among the natural sweeteners in the NIR spectra region (8000–4000 cm^{-1}). The NIR carbohydrate region (5200–4200 cm^{-1}) was not capable of distinguishing among the syrups despite the differences in origins (data not shown).

A PCA of the natural sweeteners based on the NIR carbohydrate region spectra was carried out; however, it was not possible to develop any models to classify or discriminate among the natural syrups with different origins or between syrups from the two *Agave* species (*A. tequilana* and *A. salmiana*).

3.2.2. Mid-infrared spectroscopy

The MIR spectra of the natural syrups over the region from 1500 to 900 cm^{-1} revealed characteristic differences among the sweeteners (Fig. 2A). The main differences appeared in the sugar region from 1185 to 950 cm^{-1} (Fig. 2B). Particularly strong absorption bands were found at 997, 1033, and 1062 cm^{-1}, which correspond to sucrose, glucose, and fructose, respectively (Kemsley et al., 1992; Tewari & Irudayaraj, 2004; Wang et al., 2010).

The *A. tequilana* syrups exhibited strong absorption in the fructose region (1061–1063 cm^{-1}), in agreement with their high fructose content. The *A. salmiana* syrups exhibited high sucrose content with strong absorption at 997 and 1054 cm^{-1} (Paradkar et al., 2003). The corn syrups showed strong absorption at 1026 and 1105 cm^{-1}, possibly due to high maltooligosaccharide content in the samples (Adt et al., 2006). The honey samples showed two

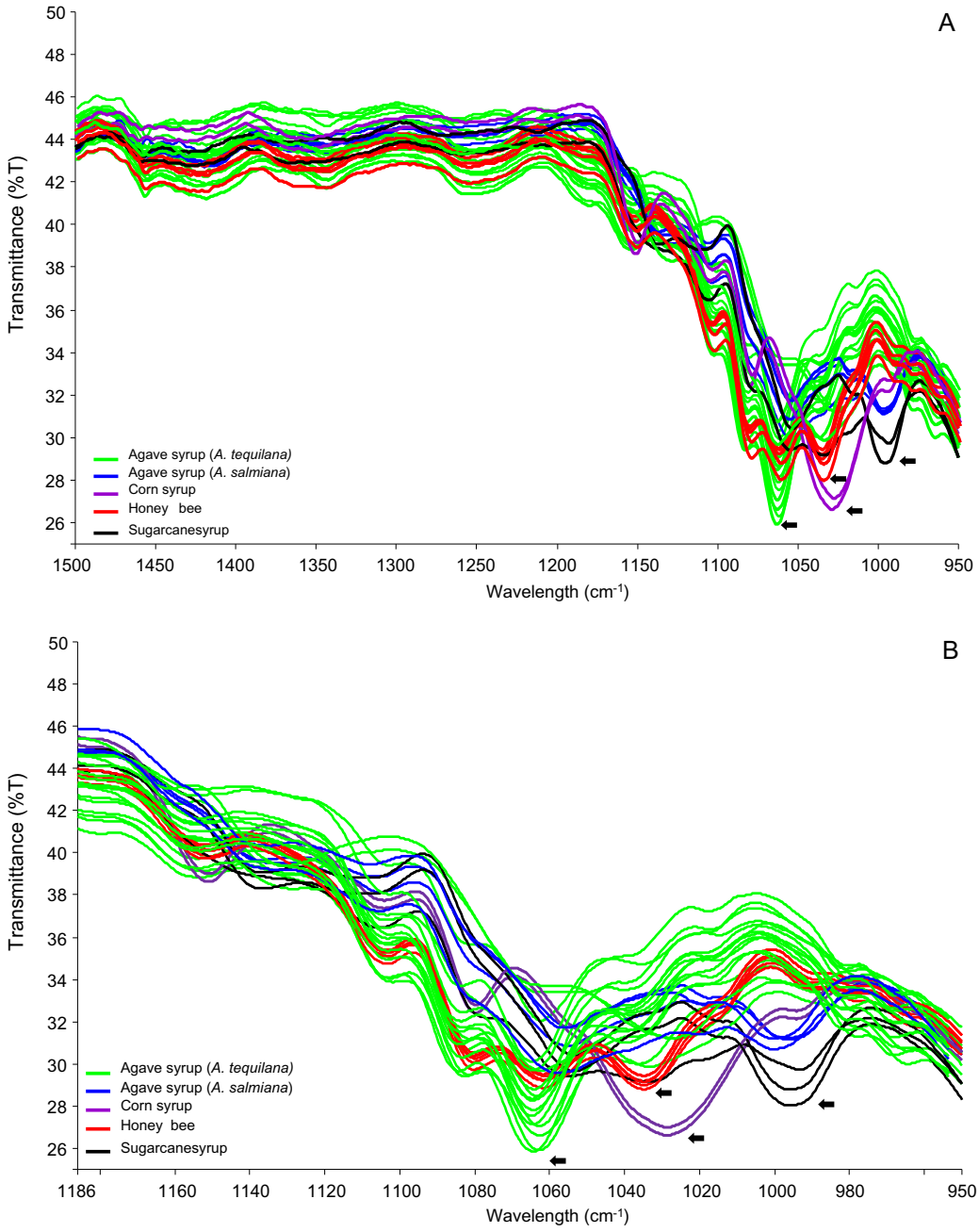

Fig. 2. MIR (%*T*) spectra of the natural syrups. (A) Region of significant variability (2600–650 cm^{-1}). (B) Enlargement of the carbohydrate region (1186–950 cm^{-1}). Arrows indicate strong absorption bands.

strong absorption regions around 1034 and 1061 cm^{-1}, corresponding to glucose and fructose, respectively. Kelly et al. (2006) described the importance of those regions for discriminating between honey and sugar syrup adulterants. The sugar cane syrups showed absorption bands at 994 cm^{-1}, due to their high sucrose content.

Principal component analysis (PCA) of the MIR spectra allowed us to identify, classify, and discriminate among the natural sweeteners according to their natural origins using the carbohydrate region (Fig. 3A and B). The *A. tequilana* syrups were grouped together and unmistakably identified among the natural syrups from different sources. Samples located away from the main agave group were related to agave syrup but probably had a different species of origin and consequently a different carbohydrate composition (e.g., samples with high fructan content; see Section 3.3.1). The *A. salmiana* syrups were grouped away from the *A. tequilana* syrups and near the sugar cane syrups (Supplementary data, Fig. 1S), probably because of the high sucrose content in the *A. salmiana* syrups and the sugar cane syrups (see Sections 3.3.1 and 3.3.2). It was possible, however, to discriminate between the *A. salmiana* syrups and the sugar cane syrups. The MIR spectra of the agave syrups revealed differences according to the source species. Because of the high carbohydrate content of both syrups, strong carbohydrate absorption bands were observed for the *A. salmiana* syrup in the sucrose region (997 cm^{-1}) and for the *A. tequilana* syrup in the fructose region (1062 cm^{-1}).

We conclude that MIR spectroscopy is a suitable tool for the identification, classification, and discrimination of natural

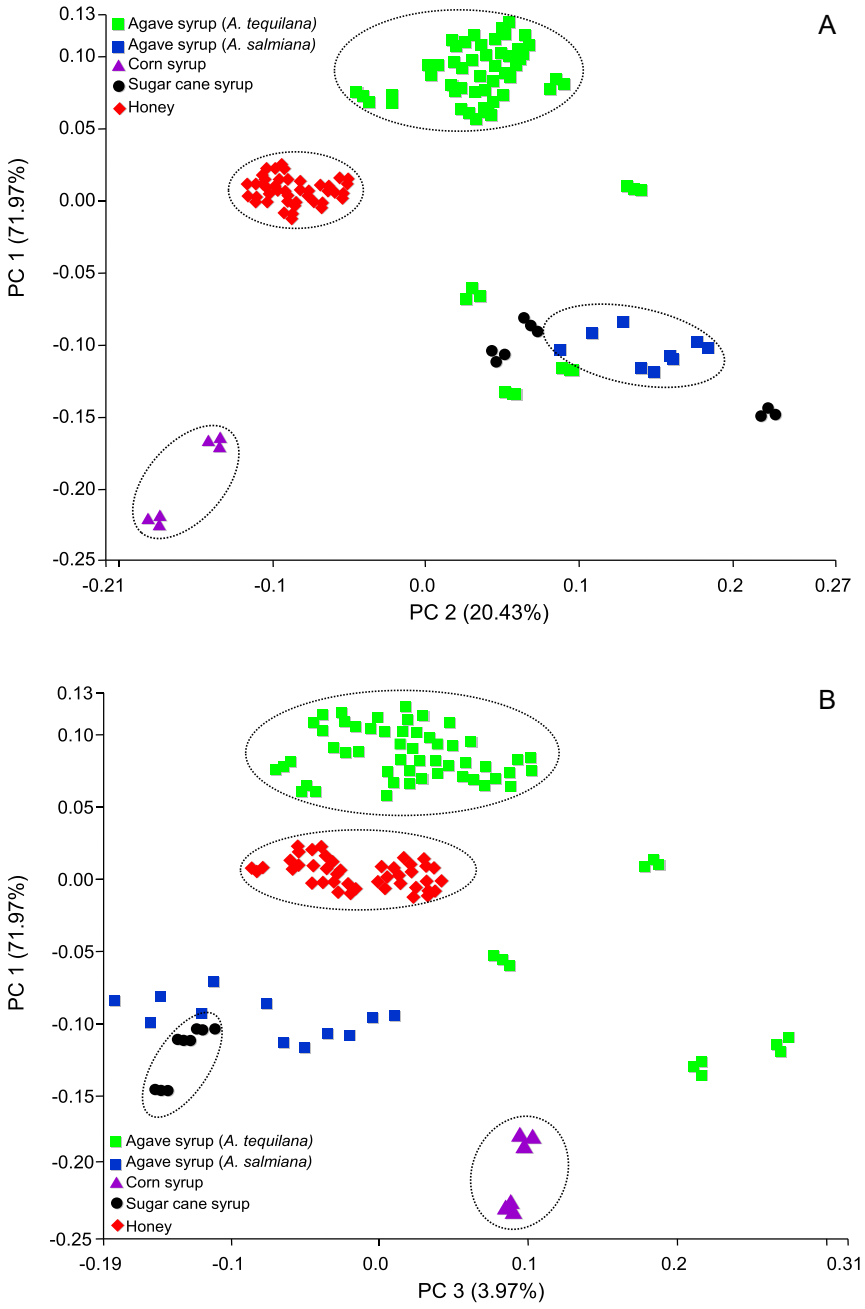

Fig. 3. Principal component analysis of the MIR carbohydrate region (1185–950 cm^{-1}) of the natural sweeteners. (A) PCA1/PCA2. (B) PCA1/PCA3.

Table 1
TLC and HPAEC-PAD of the carbohydrates identified in the natural sweeteners.

Rf[a]	Rt[b]	Nomenclature	Carbohydrate	DP[c]	Type[d]	Colour[e]
0.60	6.88	G	Glucose	1	MS	Bluish
0.60	8.00	F	Fructose	1	MS	Reddish
0.42	11.02	IM2	Isomaltose	2	MOS	Bluish
0.55	12.58	S	Sucrose	2	DS	Brown
0.25	15.20	IM3	Isomaltotriose	3	MOS	Bluish
0.44	15.92	1K	1-Kestose	3	FOS	Reddish
0.50	16.25	M2	Maltose	2	MOS	Bluish
0.52	16.73	F3	Inulotriose	3	FOS	Reddish
n.d.	17.7	6K	6-Kestose	3	FOS	Reddish
0.49	18.32	NK	Neokestose	3	FOS	Reddish
0.37	18.95	N	1-Nystose	4	FOS	Reddish
0.41	19.58	M3	Maltotriose	3	MOS	Bluish
0.31	22.12	DP5	DP5	5	FOS	Reddish
0.30	22.78	M4	Maltotetraose	4	MOS	Bluish
0.22	25.93	M5	Maltopentaose	5	MOS	Bluish
0.16	29.62	M6	Maltohexaose	6	MOS	Bluish
0.12	32.75	M7	Maltoheptaose	7	MOS	Bluish

n.d., not detected.
[a] Rf, retention factor on TLC.
[b] Rt, retention time (min) in HPAEC-PAD.
[c] DP, degree of polymerisation.
[d] ypye, DS: disaccharide, FOS: fructooligosaccharide, MOS: maltooligosaccharide, MS: monosaccharide.
[e] Colour, carbohydrate colouration in TLC.

sweeteners according to their natural source. Furthermore, MIR-SIMCA-PCA allows the classification and discrimination of syrups from different *Agave* species.

Because we were able to classify the natural sweeteners according to their origin, based on the MIR carbohydrate region, we determined the carbohydrate profile, content, and type for each of the syrups.

3.3. Carbohydrate profiles and contents of the natural sweeteners

Carbohydrate profile analysis is a valuable tool for characterising, classifying and authenticating natural syrups. It is therefore important to determine the carbohydrate profile and content of all syrups. We determined the carbohydrate profile and content of the natural sweeteners in our study by TLC and HPAEC-PAD.

3.3.1. Thin layer chromatography

The carbohydrate profiles of the natural syrups were analysed by TLC along with that of a standard mixture (Table 1). The agave syrups exhibited specific carbohydrate profiles, composed mainly of fructose (Fig. 4) and fructooligosaccharides (FOS) in most of the samples. The agave syrup samples AS9, AS11, and AS12 exhibited the lowest fructose contents, however, and showed high fructan contents, possibly due to incomplete fructan hydrolysis during the syrup elaboration process. There were differences in the carbohydrate profiles of the *A. tequilana* and *A. salmiana* syrups. Thus, the agave syrups exhibited different carbohydrate profiles according to the source species.

The agave syrups had different oligosaccharide profiles compared with the other natural sweeteners (Supplementary data, Fig. 2S). The corn syrup contained mainly maltooligosaccharides (MOS), whereas the honey contained mainly glucose and fructose, with traces of maltose, maltotriose, and other unidentified carbohydrates. Honey is made up of carbohydrates like isomaltose, nigerose, turanose, maltulose, gentiobiose, laminaribiose, panose, and others (Alvarez-Suarez, Tulipani, Romandini, Bertoli, & Battino, 2010; Cotte, Casabianca, Chardon, Lheritier, & Grenier-Loustalot, 2003), some of which may correspond to the unidentified carbohydrates observed in our results. The carbohydrate composition of sugar cane syrups is mainly glucose, fructose, and sucrose, followed by some maltooligosaccharides.

In summary, all the sweeteners had specific TLC carbohydrate profiles according to their natural source. Moreover, the TLC allowed us to differentiate between syrups from the different agave species.

3.3.2. High-performance anion exchange chromatography with a pulsed amperometric detector

3.3.2.1. Glucose, fructose, and sucrose content. The carbohydrate content and profile of the natural sweeteners were determined by HPAEC-PAD along with those of a standard mixture (Table 1) according to the methods described by Mellado-Mojica and López (2012, 2013).

In accordance with the TLC results, the HPAEC-PAD indicated that glucose, fructose, and sucrose were the most abundant carbohydrates in the natural syrups. The carbohydrate composition

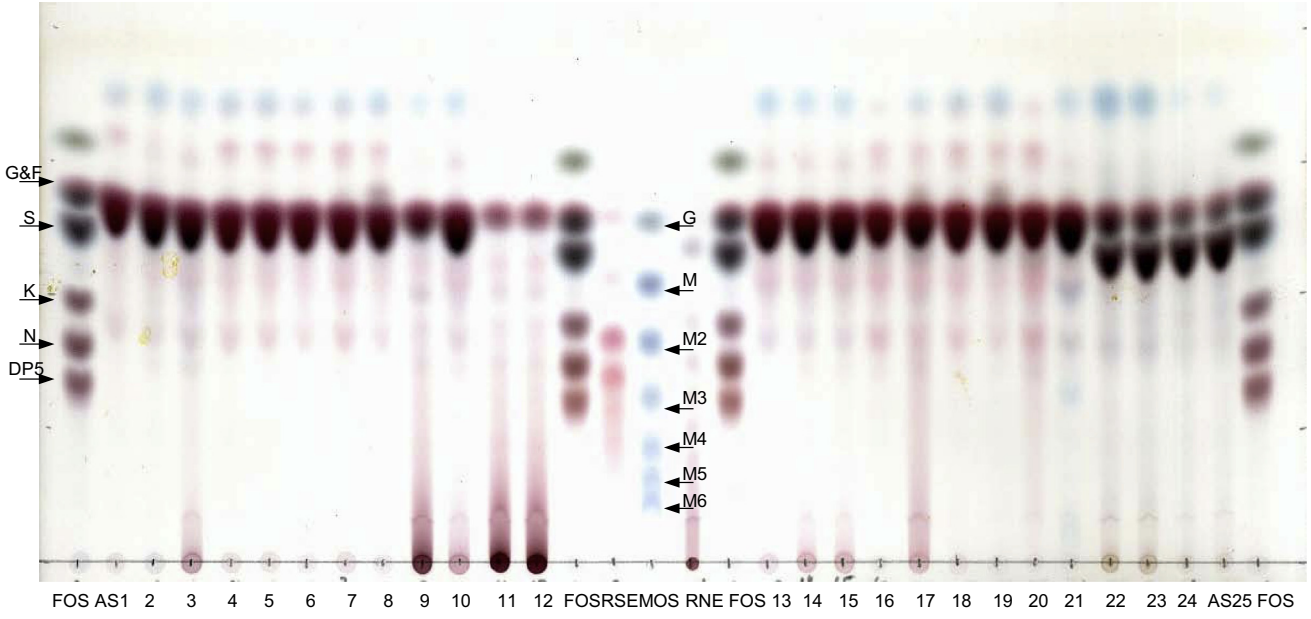

Fig. 4. Thin layer chromatography of the agave syrups (*A. tequilana* syrups: AS1–AS21. *A. salmiana* syrups: AS22–AS25). The carbohydrate nomenclature is listed in Table 1. FOS, fructooligosaccharides; RSE, raftilose; MOS, maltooligosaccharides; RNE, Raftiline.

and abundance in the syrups varied according to the source (Supplementary data, Fig. 3S). The *A. tequilana* syrups contained 24.1–689 mg g^{-1} fructose (f.w.), 1.88–186.7 mg g^{-1} glucose, and trace amounts (<8.82 mg g^{-1}) of sucrose; in some samples, including AS3, AS10, AS14–16, and AS20, FOS were determined. Although samples AS11 and AS12 exhibited the lowest fructose contents among the *A. tequilana* samples, they displayed high fructan content in the TLC analysis, suggesting that the low fructose content resulted from incomplete fructan hydrolysis during the syrup production. The *A. salmiana* syrups were mainly composed of sucrose (288–317 mg g^{-1}) followed by a smaller proportion of fructose. Thus, we determined that there were significant differences in the carbohydrate contents of the agave syrups according to the species.

The corn syrups contained from 153 to 185 mg g^{-1} glucose, although the TLC analysis showed that the corn syrup mostly contained MOS. The honey contained similar amounts of glucose (269–382 mg g^{-1}) and fructose (342–396 mg g^{-1}) and only trace amounts (4.5–31.6 mg g^{-1}) of sucrose. The sugar cane syrups were characterised by their high sucrose content (290–516 mg g^{-1});

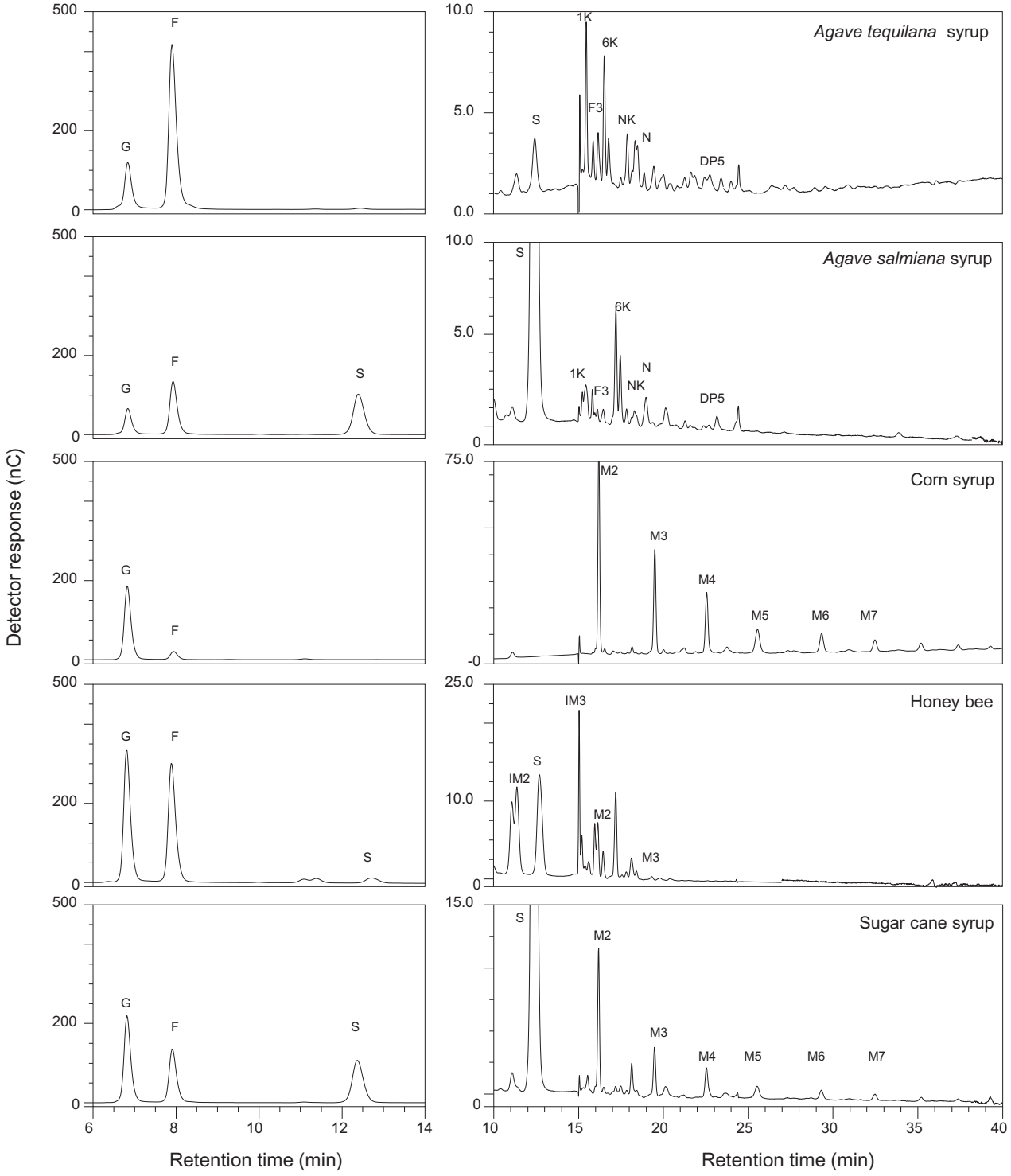

Fig. 5. HPAEC-PAD profiles of the natural sweeteners. The carbohydrate nomenclature is listed in Table 1.

however, in addition to sucrose and glucose, the sugar cane syrups contained a large amount of MOS.

The F/G ratio is an indirect measure of sweetening capacity. We used the F/G ratio to determine differences among the natural sweeteners (Supplementary data, Fig. 4S). The agave syrups had the highest F/G ratios (1.77–21.77), while the corn and sugar cane syrups had the lowest. Thus, the agave syrups displayed a higher sweetening capacity compared with the other natural sweeteners. In fact, agave syrups were described as sweeter than corn, honey, and sugar cane syrups in a sensorial analysis (Data not shown). Our results suggest that the F/G ratio could be used as a marker of the authenticity or adulteration of agave syrups (Guler et al., 2007; Ischayek & Kern, 2006; Mellado-Mojica & López, 2013).

3.3.2.2. Oligosaccharide profiles. The HPEAC-PAD profiles of the natural syrups revealed different oligosaccharide contents according to the natural origins of the syrups (Fig. 5). The *A. tequilana* syrups were dominated by fructose, glucose in a minor proportion, and FOS, such 1-kestose (1K), inulotriose (F3), 6-kestose (6K), neokestose (NK), 1-nystose (N), DP5, DP6, and DP7 with fructan traces. The *A. salmiana* syrups contained similar proportions of fructose and sucrose, as well as small quantities of FOS (1K, F3, 6K, and NK).

In addition to their high glucose content, the corn syrups contained MOS from maltose to maltoheptaose (G2–G7). The honey displayed equal proportions of glucose and fructose along with isomaltose (I2), isomaltotriose (I3), maltotose (G2), maltotriose (G3) and other unidentified peaks, which could not be confirmed as carbohydrates with the standards used but could correspond to several disaccharides and trisaccharides such as maltulose, turanose, and laminaribiose that have been reported in the literature (Alvarez-Suarez et al., 2010; Cotte et al., 2003). The sugar cane syrups were mainly composed of glucose, fructose, and sucrose with minor amounts of MOS (G2–G7) and isomaltotriose (I2) along with four unidentified peaks.

The carbohydrate profiles allowed us to validate the authenticity, origin, purity, and quality of the agave syrups. The presence of small amounts of MOS or differences in the F/G ratio in agave syrups can be interpreted as adulteration by either corn syrup or sugar cane syrup.

4. Conclusion

The physicochemical properties of the natural sweeteners we tested are very similar and cannot be used to classify or distinguish between agave syrups and other natural syrups. The physicochemical properties of different agave syrups vary according to the source species (*A. tequilana* or *A. salmiana*), however. MIR spectroscopy allowed the classification and discrimination of agave and other natural syrups. MIR spectroscopy in combination with PCA (SIMCA) can therefore be used as a fast, low cost, simple, and non-destructive tool for the classification and discrimination of syrups from different natural sources. Moreover, MIR-SIMCA-PCA has great potential for the identification, classification, and discrimination of syrups from different agave species.

TLC and HPAEC-PAD analyses showed that glucose, fructose, and sucrose were the most abundant carbohydrates in all the natural sweeteners. In addition, the F/G ratio in the agave syrups could be used to determine authenticity.

The carbohydrate profiles of the natural syrups revealed different oligosaccharide types and contents according the natural source of the sweetener. Most of the *A. tequilana* syrups had a specific carbohydrate profile composed mainly of fructose and fructooligosaccharides, whereas sucrose was the major component of the *A. salmiana* syrups. Therefore, the carbohydrate profile of agave syrups can be used as a marker for authenticity.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2014.06.111.

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